

Glycolytic Enzyme Enolase

The detailed understanding of three-dimensional protein structure is important in the design of new drugs and for engineering proteins with improved properties for industrial applications. X-ray diffraction is the most powerful method to determine the structure of such large molecules but can be applied *only* when suitable crystals are obtained. Unfortunately, not all proteins that are produced can crystallize readily and we need to identify the possible problems and try to find a reasonable rationale for solving them.

Recombinant proteins with homogeneous quaternary structures crystallize with higher probability and give more reproducible results in biochemical assays than inhomogeneous recombinant proteins. Dynamic light scattering experiments allow us to identify substrate and analogs, which are amenable to crystallization. Ease of crystal growth and diffraction quality for a particular protein is *highly* consistent with the apparent monodispersity of the complex in solution, as judged by the DynaPro® dynamic light scattering.

In this experiment we analyzed the monodispersity of several batches of purified glycolytic enzyme enolase before setting crystallization experiments at 20°C. This procedure involves expression, purification, and concentration of peak fractions followed by characterization of the enzyme in solution by the DynaPro® dynamic light scattering instrument.

This non-invasive, non-destructive technique has several advantages. For example, the experimental duration is short—suitable for routine measurements—and the sample can be recovered. We found a *direct* correlation between the monodispersity of the sample and the probability of obtaining good quality crystals in our experiments. In addition, we were able to optimize purification processes based on the polydispersity of the final product.

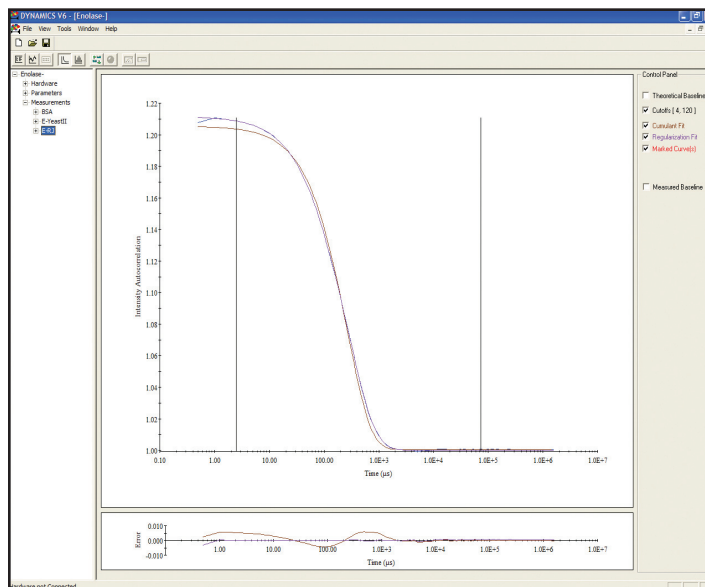


Figure 1. The DYNAMICS screen capture of the correlation function for the enolase sample.

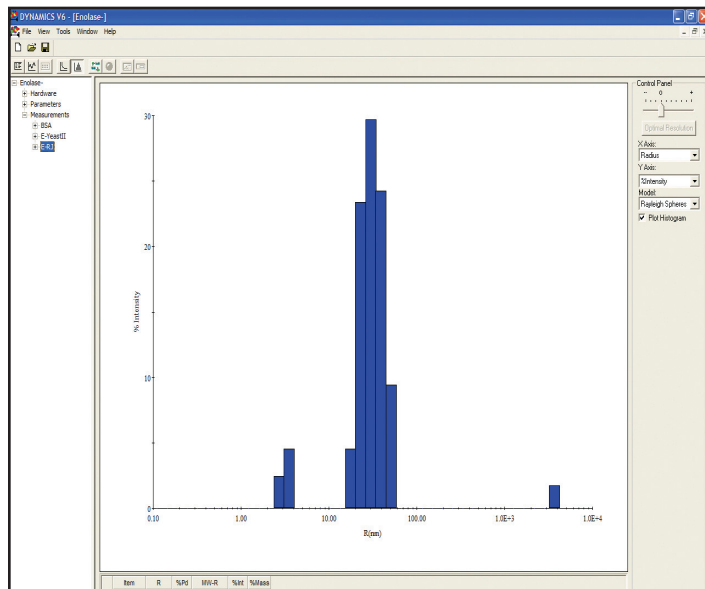


Figure 2. The DYNAMICS screen capture of the regularization view for the enolase sample.

